Cellular Dynamics international
a FUJIFILM company

iCell® Hematopoietic Progenitor Cells Prototype User's Guide

DOCUMENT #DX21748

Trademarks

iCell and MyCell are registered trademarks, and Cellular Dynamics and the  logo are trademarks of Cellular Dynamics International, Inc. and may not be used without the express written permission of Cellular Dynamics International, Inc. (CDI).

All other brands, product names, company names, trademarks, and service marks are the properties of their respective owners.

Restrictions and Liabilities

This document is provided "as is." CDI assumes no responsibility for any typographical, technical, or other inaccuracies in this document. CDI reserves the right to periodically change information that is contained in this document; however, CDI makes no commitment to provide any such changes, updates, enhancements, or other additions to this document to you in a timely manner or at all.

THE iCELL HEMATOPOIETIC PROGENITOR CELLS ARE PROVIDED "AS IS" TO YOU. TO THE FULLEST EXTENT PERMITTED BY APPLICABLE LAW, CDI MAKES NO, AND DISCLAIMS ALL, REPRESENTATIONS, WARRANTIES, CONDITIONS OR COVENANTS, EXPRESS OR IMPLIED, WITH RESPECT TO ANY PRODUCT REFERENCED HEREIN OR PERFORMANCE OF ANY SERVICES REFERENCED HEREIN, INCLUDING BUT NOT LIMITED TO, ANY EXPRESS OR IMPLIED WARRANTIES OR CONDITIONS OF FITNESS FOR A PARTICULAR PURPOSE, NON-INFRINGEMENT, MERCHANTABILITY, DURABILITY, TITLE, OR RELATED TO THE PERFORMANCE OR NON-PERFORMANCE OF ANY PRODUCT REFERENCED HEREIN OR PERFORMANCE OF ANY SERVICES REFERENCED HEREIN).

This document might contain references to third-party sources of information, hardware or software, products or services and/or third-party websites (collectively the "Third-Party Information"). CDI does not control, and is not responsible for, any Third-Party Information, including, without limitation the content, accuracy, copyright compliance, compatibility, performance, trustworthiness, legality, decency, links, or any other aspect of Third-Party Information. The inclusion of Third-Party Information in this document does not imply endorsement by CDI of the Third-Party Information or the third party in any way.

CDI does not in any way guarantee or represent that you will obtain satisfactory results from using iCell Hematopoietic Progenitor Cells as described herein. You assume all risk in connection with your use of iCell Hematopoietic Progenitor Cells.

Conditions of Use

iCell Hematopoietic Progenitor Cells are for life science research use only and subject to the use restrictions contained in Appendix A. You are responsible for understanding and performing the protocols described within this guide. CDI does not guarantee any results you may achieve. These protocols are provided as CDI's recommendations based on its use and experience with iCell Hematopoietic Progenitor Cells.

Origin

iCell Hematopoietic Progenitor Cells are manufactured in the United States of America.

Copyright Notice

Revision History

Document #DX21748
Version 0.2: February 2017
Version 0.1: December 2014

Table of Contents

Before You Begin

Notes

- Immediately transfer the frozen vials to liquid nitrogen storage.
- Read this entire iCell® Hematopoietic Progenitor Cells Prototype User's Guide before handling or using iCell Hematopoietic Progenitor Cells.
- iCell Hematopoietic Progenitor Cells are for life science research use only. See Appendix A for more information and other restrictions.
- A Safety Data Sheet (SDS) for dimethyl sulfoxide (DMSO), in which iCell Hematopoietic Progenitor Cells are frozen, is available online at www.cellulardynamics.com/lit/ or on request from Cellular Dynamics International. Only technically qualified individuals experienced in handling DMSO and human biological materials should access, use, or handle iCell Hematopoietic Progenitor Cells.

Notes

Chapter 1. Introduction

Cellular Dynamics International's (CDI) iCell Hematopoietic Progenitor Cells are a highly pure population of human multipotent hematopoietic progenitor cells (HPCs) derived from induced pluripotent stem (iPS) cells using CDI's proprietary differentiation and purification protocols. iCell Hematopoietic Progenitor Cells express the glycoprotein CD34 on the cell surface and are capable of generating in vitro blood cells of different lineages. As iCell Hematopoietic Progenitor Cells are maintained in culture and differentiated into hematopoietic lineages, the levels of CD34 expression decrease, and the expression of lineage specific markers is acquired. These cells provide a reliable source of human HPCs suitable for use in targeted drug discovery, toxicity testing, and other life science research.

When thawed and plated as recommended in this User's Guide, iCell Hematopoietic Progenitor Cells retain their proliferative capacity and broad developmental potential. A supplementation of a commercially available HPC Culture Medium with selected combinations of cytokines and growth factors can drive the differentiation of iCell Hematopoietic Progenitor Cells into multiple lineages of the hematopoietic system including erythrocytes, granulocytes, and monocytes/macrophages. Given the transient nature of self-renewal and multipotency, iCell Hematopoietic Progenitor Cells should be used in proliferation and differentiation assays immediately after thawing.

Components Supplied by Cellular Dynamics

Item	Catalog Number
iCell Hematopoietic Progenitor Cells Prototype[1]	HPC-301-020-001-PT
iCell Hematopoietic Progenitor Cells Prototype User's Guide[1]	
Certificate of Testing[2]	
Certificate of Origin If required for shipping purposes	

1 Safety Data Sheet and User's Guide available online at www.cellulardynamics.com/lit/

2 Available online at www.cellulardynamics.com/cot/

Required Equipment and Consumables

Item	Vendor	Catalog Number
Equipment		
37°C Water Bath	Multiple Vendors	
Biological Safety Cabinet with UV Lamp	Multiple Vendors	
Cell Culture Incubator	Multiple Vendors	
Hemocytometer or Automated Cell Counter[1]	Multiple Vendors	
Liquid Nitrogen Storage Unit	Multiple Vendors	
Pipettors	Multiple Vendors	
Tabletop Centrifuge	Multiple Vendors	
Tube Shaker	Multiple Vendors	
Consumables		
15 ml and 50 ml Centrifuge Tubes	Multiple Vendors	
D-PBS	Life Technologies	14190
Ethanol	Multiple Vendors	
FBS	Hyclone	SH30396.03
HPC Culture Medium[2]	Multiple Vendors	
IMDM	Life Technologies	12440
PES Filter Unit, 0.2 µm, 150 ml	Multiple Vendors	
Pipettes	Multiple Vendors	
Poly(2-hydroxyethyl methacrylate) (Poly-HEMA)	Sigma	P3932
Sodium Hydroxide (NaOH)	Multiple Vendors	
Sterile Tissue Culture Grade Distilled Water	Multiple Vendors	
Ultra Low Attachment Cell Culture Vessels	Corning	3815 (T25 Flask)
Untreated Cell Culture Vessels	Corning	431463 (T25 Flask)

1 Ensure the automated cell counter is appropriately calibrated before use.

2 Recommended HPC Culture Medium include StemPro-34 SFM (Life Technologies, Cat. No. 10640-019, prepared according to the manufacturer's instructions) or StemSpan SFEM (Stem Cell Technologies, Cat. No. 09650). HPC Culture Medium should be supplemented with hematopoietic growth factors and cytokines as required for intended use.

Notes

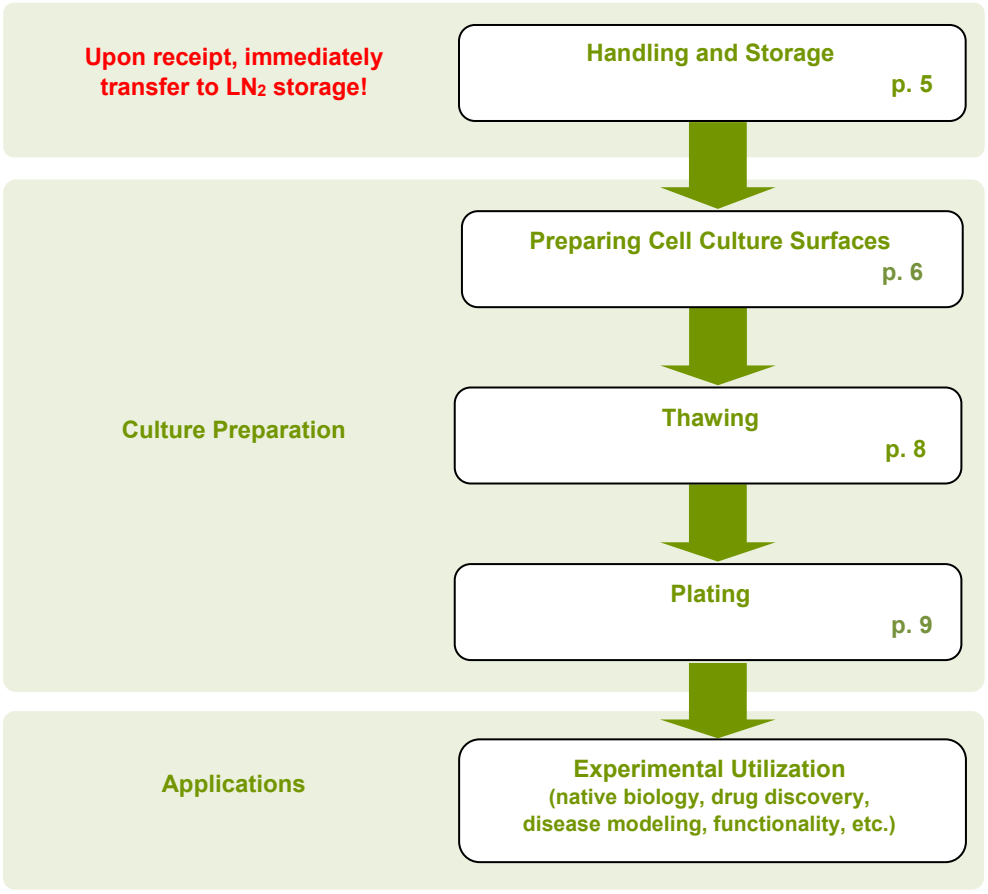
Notes

Technical Support and Training

CDI's Technical Support Scientists have the necessary laboratory and analytical experience to respond to your inquiries. In addition, in-lab training may be available upon request.

Telephone	(877) 320-6688 (US toll-free) / (608) 310-5100 x5 Monday - Friday, 8:30 am - 5:00 pm US Central Time
Fax	(608) 310-5101
Email	support@cellulardynamics.com

Workflow Diagram



Notes

Notes

Chapter 2. Handling and Storage

iCell Hematopoietic Progenitor Cells are provided as cryopreserved single-cell suspensions in 1.5 ml cryovials. Upon receipt, directly transfer the cryobox containing iCell Hematopoietic Progenitor Cells to the vapor phase of a liquid nitrogen storage dewar. CDI strongly recommends transferring the entire cryobox into the storage rack to avoid transferring individual vials.

It is critical to maintain cryopreserved iCell Hematopoietic Progenitor Cells at a stable temperature. Minimize exposure of cryopreserved iCell Hematopoietic Progenitor Cells to ambient temperature when transferring vials to liquid nitrogen storage.

Chapter 3. Preparing Cell Culture Surfaces

Notes

iCell Hematopoietic Progenitor Cells will function optimally in the following cell culture vessels:

- Ultra Low Attachment cell culture vessels
- Freshly coated cell culture vessels with poly-HEMA as described below

Preparing the Poly-HEMA Cell Culture Vessel

The following procedure details coating eight untreated T25 flasks. Scale volumes appropriately for other vessel formats.

1. Prepare a 95% ethanol solution by mixing the following components in a 50 ml centrifuge tube at the volumes specified:

Component	Volume/40 ml	Final Concentration
Ethanol, 100%	38 ml	95%
NaOH, 1M	400 µl	10 mM
Sterile Tissue Culture Grade Distilled Water	1.6 ml	N/A

2. Add 4 g of poly-HEMA to the ethanol solution and immediately invert the centrifuge tube to prevent clumping.
3. Shake the tube at room temperature overnight.
4. Add 5 ml of poly-HEMA solution to each flask and rotate until all sides are coated, avoiding the cap.
5. Aspirate poly-HEMA solution from the flasks and let dry with the caps off in a biological safety cabinet overnight. It is recommended to aspirate the flasks 2 times to ensure complete removal of the poly-HEMA solution.
6. Rinse each flask with 5 ml of D-PBS before plating iCell Hematopoietic Progenitor Cells.

   Note: *If necessary, store the flasks at 4°C for up to 1 week.*

Notes

Chapter 4. Preparing the Thawing Medium

iCell Hematopoietic Progenitor Cells thawing requires preparation of the Thawing Medium. Prepare using sterile technique and store as follows:

1. Mix the following components at the volumes specified:

Component	Volume/100 ml	Final Concentration
IMDM	90 ml	90%
FBS	10 ml	10%

2. Filter the Thawing Medium using a 150 ml, 0.2 µm PES filter unit.
3. Store the Thawing Medium at 4°C, protected from light, for up to 1 month.

Chapter 5. Thawing iCell Hematopoietic Progenitor Cells

Notes

Maintain iCell Hematopoietic Progenitor Cells in liquid nitrogen until immediately before thawing to ensure maximal performance of the cells. Complete the following steps of the thawing procedure in a time-efficient manner to facilitate optimal iCell Hematopoietic Progenitor Cells viability and performance.

Note: *Thaw no more than 1 vial at one time.*

1. Equilibrate the Thawing Medium and HPC Culture Medium at room temperature for 2 - 4 hours before thawing iCell Hematopoietic Progenitor Cells.
2. Remove the frozen iCell Hematopoietic Progenitor Cells cryovial from the liquid nitrogen storage tank.

   Note: *If necessary, place cryovials on dry ice for up to 10 minutes before thawing.*
3. Immerse the cryovial in a 37°C water bath (avoid submerging the cap) and gently swirl for 2 minutes.
4. Immediately remove the cryovial from the water bath, spray with 70% ethanol, and place in a biological safety cabinet.
5. Gently transfer the iCell Hematopoietic Progenitor Cells cryovial contents to a 50 ml centrifuge tube using a 1 ml pipettor.

   Note: *Use of a 50 ml centrifuge tube facilitates suitable mixing to minimize osmotic shock and increase viability.*
6. Rinse the empty iCell Hematopoietic Progenitor Cells cryovial with 1 ml of room temperature Thawing Medium to recover any residual cells from the vial. Transfer the 1 ml of medium rinse from the cryovial drop-wise (~1 drop/second) to the 50 ml centrifuge tube containing the iCell Hematopoietic Progenitor Cells suspension. Gently swirl the tube while adding the medium to mix the solution completely and minimize the osmotic shock on the thawed cells.

   Drop-wise addition of the Thawing Medium to the cell suspension is critical to minimize osmotic shock and ensure maximum viability of the cells.
7. Slowly add 8 ml of room temperature Thawing Medium to the 50 ml centrifuge tube (~1 - 2 drops/second). Gently swirl the centrifuge tube while adding the medium.

   It is critical to add the 8 ml of Thawing Medium slowly to ensure maximum viability of the cells.
8. Centrifuge the cell suspension at 300 x g for 5 minutes at room temperature.
9. Aspirate the supernatant from the 50 ml centrifuge tube being careful not to disturb the cell pellet.
10. Resuspend iCell Hematopoietic Progenitor Cells in 5 ml of HPC Culture Medium.

Notes

Chapter 6. Plating iCell Hematopoietic Progenitor Cells

Use iCell Hematopoietic Progenitor Cells in proliferation or differentiation assays immediately after thawing. Supplement the HPC Culture Medium with the appropriate cytokines and growth factors combinations to drive proliferation or differentiation into the lineage of choice.

1. Remove a sample of cells to perform a cell count using a hemocytometer (using trypan blue exclusion to identify viable cells) or an automated cell counter.
2. Dilute the cell suspension using room temperature HPC Culture Medium to obtain a desired cell plating density.
3. Culture iCell Hematopoietic Progenitor Cells in a cell culture incubator at 37°C, 5% CO_2.

Expected Cell Density

iCell Hematopoietic Progenitor Cells can be plated at various densities to accommodate different application needs. The thawing procedure will result in a viable cell concentration of approximately 2 x 10^5 cells/ml in 5 ml of total volume per vial. Figure 1 shows the expected density that can be obtained by plating 10 x 10^5 cells in a T25 flask.

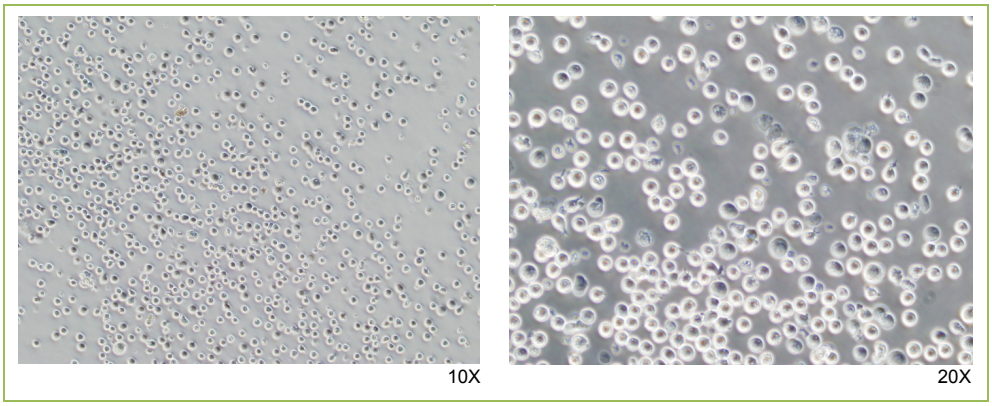


Figure 1: iCell Hematopoietic Progenitor Cells at 24 Hours Post-plating
These images show the expected morphology of iCell Hematopoietic Progenitor Cells.

Appendices

Notes

Appendix A. Intellectual Property Rights, Use Restrictions, and Limited License

A. **OWNERSHIP.** The Products are covered by pending patents and patents: cellulardynamics.com/about-us/patents/. Customer has a limited license to use the Products for internal research purposes for the sole benefit of the Customer, subject to the use restrictions and third party licenses included in subsection B of this Appendix A. Customer acknowledges and agrees that the receipt or purchase of the Products by Customer shall not be construed as a transfer of any title or the grant of any rights in or to the intellectual property embodied in the Products owned or licensed by Cellular Dynamics. In particular, no right or license to make, have made, offer to sell, or sell the Products, to modify or reproduce the Product or any part thereof, or to use the Products in combination with any other product(s), except product(s) provided or expressly licensed to Customer by Cellular Dynamics for such use, is implied or conveyed by the sale or transfer of Products to Customer.

B. **USE RESTRICTIONS.** The Products are licensed for internal research purposes only, and may not be used for any other purpose. The Products must be used in accordance with this User's Guide to which Customer, by ordering and accepting the Products, agrees. Customer shall not make, have made, offer to sell, or sell the Products. Unless expressly permitted in a contract executed between Customer and Cellular Dynamics, Customer shall not use the Products (or any modifications Customer makes to the Products or any cells derived, developed or expanded from the Products) in (i) the manufacture of any products, or (ii) any services for a third party. Customer may not transfer the Products (or any modifications Customer makes to the Products or any cells derived, developed or expanded from the Products) to any third party without Cellular Dynamics' prior written consent. Customer shall not reverse engineer the Products. Customer shall not use the Products, components or modifications thereof, or any cells derived, developed or expanded therefrom, in humans, in clinical trials, for diagnostic purposes involving human subjects, or for any investigational or other therapeutic use. Customer shall not use the Products directly or indirectly to derive or make any human gamete or gamete precursor cell. Customer shall use the Products in accordance with all applicable laws and regulations and any applicable institutional review board approved protocol and/or privacy office approval. Customer is not entitled to receive any data or information from Cellular Dynamics that directly identifies the donor of the biological materials from which the Products indirectly are derived or were made. Customer shall not attempt in any way to determine the identity of the donor of the biological materials from which the Products indirectly are derived or was made.

C. **DATA.** Customer agrees that if described on Customer's product quotation from Cellular Dynamics it will provide data and information as described therein to Cellular Dynamics regarding Customer's use of the Products.

Appendix B. Product Provided "AS IS"

A. The Products are sold or provided "AS IS."

B. TO THE FULLEST EXTENT PERMITTED BY APPLICABLE LAW, CELLULAR DYNAMICS DISCLAIMS, ALL REPRESENTATIONS, AND WARRANTIES, EXPRESS OR IMPLIED (INCLUDING BUT NOT LIMITED TO, ANY IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE OR NON-INFRINGEMENT), AND ALL LIABILITY, WITH RESPECT TO THE PRODUCTS; AND BY ORDERING AND ACCEPTING THE PRODUCTS CUSTOMER WAIVES ALL RIGHTS AND REMEDIES OTHERWISE AVAILABLE WITH RESPECT TO THE PRODUCTS.

Notes

C. Customer will be solely responsible for (i) Customer's use of the Products for a purpose or in a manner other than that for which they were designed or that is permitted or in breach of the Use Restrictions above; (ii) Customer's failure to follow this User's Guide for the use, storage, and handling of the Products however such failure is caused; (iii) Customer's failure to comply with any of the provisions of Appendix A above; and (iv) any abuse, other misuse or neglect of the Products by Customer or any damage or loss of the Products by events or occurrences beyond a person's (e.g., Cellular Dynamics') control including without limitation, accident, fire, vandalism and natural disasters (acts of God).

D. Customer acknowledges and agrees that Cellular Dynamics may fill Customer's order with any number of units of Products. Such units may be more units than Customer ordered. Customer will not be charged extra for any adjustments made by Cellular Dynamics. Because the number of cells in a unit may vary from lot to lot, Cellular Dynamics reserves the right to fill the order with that number of units which is sufficient to fill Customer's order.

Appendix C. Limited Liability

TO THE FULLEST EXTENT PERMITTED UNDER APPLICABLE LAW, CELLULAR DYNAMICS SHALL NOT HAVE ANY LIABILITY FOR INCIDENTAL, COMPENSATORY, PUNITIVE, CONSEQUENTIAL, INDIRECT, SPECIAL OR OTHER SIMILAR DAMAGES, HOWEVER CAUSED AND REGARDLESS OF FORM OF ACTION WHETHER IN CONTRACT, TORT (INCLUDING NEGLIGENCE), STRICT PRODUCT LIABILITY OR OTHERWISE, EVEN IF CELLULAR DYNAMICS HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. NOTWITHSTANDING ANY OTHER TERM OR IMPLICATION TO THE CONTRARY, UNDER NO CIRCUMSTANCES SHALL CELLULAR DYNAMICS' LIABILITY TO CUSTOMER EXCEED THE AMOUNT PAID BY CUSTOMER FOR THE PRODUCTS TO CELLULAR DYNAMICS.